Statement on the safe use of the nptII antibiotic resistance marker gene in genetically modified plants by the Scientific Panel on genetically modified organisms (GMO)

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Statement of the Scientific Panel on Genetically Modified Organisms on the safe use of the *npt*II antibiotic resistance marker gene in genetically modified plants

adopted on 22-23 March 2007

1. BACKGROUND

The Commission is currently considering the authorisation for placing on the market of the genetically modified (GM) potato line EH92-527-1 under Regulation (EC) No 1829/2003 on GM food and feed and under Directive 2001/18/EC, part C. The GM potato, developed for amylopectin production, also contains a *npt*II gene used as a selectable marker. The *npt*II gene codes for an aminoglycoside phosphotransferase conferring resistance to antibiotics such as kanamycin, neomycin, paromomycin, butirosin, gentamycin B and geneticin.

Directive 2001/18/EC (EC, 2001) states that Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment. This is with a view to identify and phase out antibiotic resistance marker genes (ARMGs) in GMOs which may have adverse effects on human health and the environment.

Over the last years, the use of antibiotic resistance marker genes for selection of GM plants, and which have been subject of safety assessment under Part C of the Directive 2001/18/EC, has been limited to the *npt*II gene. Some applications of GM plants submitted under Regulation No 1829/2003 also include the use of the *npt*II gene as a selectable marker, while in other cases the *npt*II gene has been excised after selection, or other markers such as the *epsps* and *pat* genes coding for herbicide tolerance were used. No other antibiotic resistance marker genes are currently present in applications submitted for approval.

According to an earlier conclusion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) (EFSA, 2004)[1] the use of *npt*II as a selectable marker in genetically modified plants and, more specifically, in the potato line EH92-527-1 (EFSA, 2006), does not pose a risk to the environment or to human and animal health. This conclusion was based on the low probability of gene transfer from plants to bacteria, the already widespread presence of the *npt*II gene in bacterial populations, and the limited use of kanamycin and neomycin in human and veterinary medicine.

The Commission sought confirmation from the European Medicines Agency (EMEA) as to whether, notwithstanding a WHO working group classification of aminoglycosides as critically important antibacterials (WHO, 2005), the current or possible future uses of the antibiotics for which the *npt*II gene

[1]http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/384.Par.0001.File.dat/opinion_gmo_05_en1.pdf

confers resistance is still in line with the earlier EFSA opinion. The EMEA was asked to consider whether the current or possible future medicinal uses of these antibiotics might have an impact on the earlier conclusions of the GMO Panel.

In response to the Commission's request, the EMEA indicated that aminoglycosides comprise a class of antibiotics that has become increasingly important in the prevention and treatment of serious invasive bacterial infections in humans, since Gram-negative bacteria (and tuberculosis bacteria) are becoming resistant to other classes of antibiotics. The EMEA also stressed that, although kanamycin and neomycin are used relatively infrequently, the potential development of new chemical entities similar to kanamycin and neomycin should also be taken into account. In addition, although the veterinary use of kanamycin and neomycin is currently limited, aminoglycosides as a group are a class of antibiotics critically important for veterinary medicine.

The EMEA considered that its competence did not extend to a detailed consideration of the likelihood of gene transfer of antibiotic resistance genes from plant material to bacteria of man and animals.

Subsequently, the Commission requested EFSA (letter dated March 2, 2007) to consider the information provided by the EMEA and to indicate the potential consequences of the EMEA's conclusions on the safety assessment of the *npt*II gene and, where applicable, on the specific assessments of GMOs and derived food and feed.

2. ASSESSMENT

A concern with respect to the presence of antibiotic resistance marker genes in GM plants is the potential for increased resistance to antibiotics in humans, animals and in organisms in the wider environment as a result of horizontal gene transfer. The safety assessment of the GMO Panel concerning the presence of the *npt*II gene in GM plants builds on a number of considerations. Key elements are the very low likelihood of transfer of a functional *npt*II gene (or any other gene), from GM plant material to microorganisms, and the prevalence of the *npt*II gene in bacterial clinical isolates and in the environment.

2.1. Likelihood of transfer of the nptII *gene from the genome of GM plants to bacteria*

In considering the probability of functional gene transfer from plants into bacteria in the environment or human/animal gut, several aspects need to be taken into account:

(i) DNA is released from plant material by normal digestion processes that take place in the gastrointestinal tract, or by activities of nucleases present in various organisms in the environment.

(ii) The probability that bacteria will be exposed to DNA stretches long enough to contain the intact *npt*II gene is very low because of the above mentioned digestion and degradation processes (Lorenz and Wackernagel, 1994).

(iii) The *npt*II gene from plant material can only be taken up by competent bacteria via natural transformation, a process that occurs infrequently in many bacteria and in most environmental conditions (Davison, 1999).

(iv) If the intact *npt*II gene enters the bacteria, it will be rapidly degraded by restriction endonucleases in many bacterial cells which possess DNA restriction systems in order to destroy foreign DNA (Davison, 1999).

(v) If the intact *npt*II gene does indeed survive, the probability of its incorporation into the bacterial genome is very low unless there are homologous regions already present in the bacterial genome. Gene transfer from plants to bacteria has only been demonstrated under laboratory conditions when regions of homology were already present in the recipient bacterium (Bennett et al., 2004, de Vries et al., 2001, de Vries and Wackernagel, 2002, Kay et al., 2002, Tepfer et al., 2003) .

(vi) Expression of the incorporated *npt*II gene is unlikely considering that in GM plant material the *npt*II gene is under the control of a promoter with preferential expression in plants, which does not support its efficient expression in bacteria.

(vii) Stable integration and inheritance of the *npt*II gene in the host bacterium is not likely in the absence of selective pressure from a relevant antibiotic.

When all of the above mentioned aspects are taken into account, the probability of functional gene transfer from plants into microorganisms is extremely low. It is not surprising that transfer of an antibiotic resistance marker from GM plants to bacteria has not been observed under natural conditions (Gay and Gillespie, 2005).

The EMEA has indicated that under laboratory conditions gene transfer from plants to bacteria has been demonstrated. EFSA has addressed this issue more extensively in its Opinion of 2004 (section 4) (EFSA, 2004). Gene transfer from plants to bacteria has only been demonstrated in a few highly transformable bacterial species (e.g., *Acinetobacter* sp. BD413 or *Pseudomonas stutzeri*) under artificial and forced laboratory conditions when regions of homology were already present in the recipient bacterium (Bennett et al., 2004, de Vries et al., 2001, de Vries and Wackernagel, 2002, Kay et al., 2002, Tepfer et al., 2003). In the absence of this optimisation of the process and selection pressure, resistance gene transfer from GM plants to bacteria, even in the laboratory, could not be demonstrated (Gebhard and Smalla, 1998).

2.2. Prevalence of the nptII *gene in soil, humans and animals*

As indicated in the Opinion (of the GMO Panel) on the use of antibiotic resistance genes as markers in GM plants, antibiotic resistance is a common feature in natural microbial communities in soils, aquatic systems, and habitats associated with animals and humans (EFSA, 2004).

There is already a widespread presence of *npt*II in the soil environment as evidenced from DNA-based work with *npt*II as a probe in different locations in Western Europe (NSCFFS, 2005, Smalla et al., 1993) and in the USA (Leff et al., 1993).

Studies indicate that, as expected of a gene located on a transposable genetic element, *npt*II is located on a wide range of replicons in bacterial clinical isolates from humans (Alvarez and Mendoza, 1992, Chang et al., 1992, Flamm et al., 1993). The *npt*II gene was present in 2.5% of bacterial clinical isolates resistant to kanamycin and neomycin collected between 1987 and 1991 in several European and Central and South American countries (Shaw et al., 1993). Studies on the prevalence of the *npt*II gene in animal-associated bacterial populations have not been found in the scientific literature.

2.3. Contribution of the nptII *gene to the prevalence of resistance to kanamycin*

Kanamycin-resistant bacteria are ubiquitous in nature. Selective plating of different environmental samples on kanamycin-containing medium reduced the microbial count from 10^7 to 10^4 CFU/g (Smalla and van Elsas, 1996, Smalla et al., 1993). Only a fraction of kanamycin-resistant bacteria contain the *npt*II (*aph(3')-IIa*) gene, the other resistant bacteria having different genes and/or other mechanisms conferring kanamycin resistance. The *npt*II gene has been reported to occur naturally only in eubacteria. In one survey, 3 out of 184 kanamycin resistant bacterial isolates from three stream sites in the USA (Leff et al., 1993) and 44 out of 355 from different habitats in the Netherlands (Smalla et al., 1993) contained *npt*II sequences.

2.4. Potential mutations of the nptII *gene resulting in resistance to other antibiotics*

As reported in the opinion of the GMO Panel on antibiotic resistance genes as markers in GM plants (EFSA, 2004), resistance towards amikacin, an important reserve antibiotic could be obtained under laboratory conditions and was the result of a mutated *npt*II gene and a diminished rate of amikacin uptake into the bacterial cell (Perlin and Lerner, 1986). The increased affinity of a mutated *npt*II gene product for amikacin was later confirmed by site-directed mutagenesis which resulted in one altered nucleotide in the gene and an eight-fold increase in amikacin resistance in *E.coli* (Kocabiyik and Perlin, 1992). It has been suggested that the increased affinity for amikacin conferred by this mutation, might impair the clinical effectiveness of the drug. However, to date no clinical amikacin resistant strains with a mutated *npt*II gene have been identified.

3. CONCLUSIONS

The GMO Panel agrees with the EMEA that the preservation of the therapeutic potential of the aminoglycoside group of antibiotics is important. The Panel is also of the opinion that the therapeutic effect of these antibiotics will not be compromised by the presence of the *npt*II gene in GM plants, given the extremely low probability of gene transfer from plants to bacteria and its subsequent expression. Furthermore, the GMO Panel considers it very unlikely that the presence of the *npt*II gene in GM plants will change the existing widespread prevalence of this antibiotic resistance gene in bacterial sources in the environment. The GMO Panel also points to evidence which indicates that integration of the *npt*II gene would only be one of many mechanisms by which bacteria could become resistant to aminoglycosides such as kanamycin.

Therefore, the GMO Panel reiterates its earlier conclusions (EFSA, 2004) that the use of the *npt*II gene as selectable marker in GM plants (and derived food or feed) does not pose a risk to human or animal health or to the environment. The GMO Panel also confirms earlier safety assessments of GM plants and derived food/feed comprising the *npt*II gene.

The GMO Panel emphasizes that the use of antibiotic resistance marker genes in GM plants has been the subject of several reviews (Gay and Gillespie, 2005, Goldstein et al., 2005, Miki and McHugh, 2004, Nap et al., 1992, Nielsen et al., 1998, Ramessar et al., 2007) and expert consultations: Working Party of the British Society for Antimicrobial Chemotherapy (Bennett et al., 2004), FAO/WHO Consultation on Foods Derived from Biotechnology (FAO/WHO, 2000), Scientific Steering Committee of the European Commission (SSC, 1999) Zentrale Kommission für die Biologische Sicherheit, DE (ZKBS, 1999), The Advisory Committee on

Novel Foods and Processes, UK (ACNFP, 1996). It has been concluded in these reports that the frequencies of gene transfer from plants to bacteria are likely to be extremely low and that the presence of antibiotic resistance marker genes, and in particular the *npt*II gene, in GM plants do not pose a relevant risk to human or animal health or to the environment.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from DG SANCO, dated 2 March 2007, concerning the presence of the *npt*II resistance gene in genetically modified organisms (ref. SANCO/E1/SG/cc (2007)D/510137).

2. Document from the EMEA Committee for Medicinal Products for Veterinary Use and Committee for Medicinal Products for Human Use, dated 22 February 2007, entitled "Presence of the antibiotic resistance marker gene nptII in GM plants for food and feed uses", (ref. EMEA/CVMP/56937/2007-Final).

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